

FIG. 1

	10	20	30	40	50
Bet v 1 sense	5'-	A A T T A T G A G A C T G A G A C C A C C T C T G G G I G G A G A C A T T A T C C C A G C C A G C T C G			
Bet v 1 non-sense	3'-	T T A A T A C T C T G A C T C T G G G I G G A G A C A T T A T C C C A G C C A G C T C G A G C			
sense primer	5'-	T G A G A C C C C C C T C T G T T A T C C C A G			
non-sense primer	3'-	A T A C T C T G A C T C T G G G A G A C A			

FIG. 2

Oligonucleotide primers for site directed mutagenesis of Bet v 1 (No. 2801).

all sense	1: 183Bv,	15-mer 5'-	G T T G C C A A C C G A T C A G
1 sense	2: 184Bv,	23-mer 5'-	T G A G A C C C C T C T G T T A T C C C A G
1 non-sense	3: 185Bv,	23-mer 5'-	A C A G A C C C C T C T C A G T C C A T A
2 sense	4: 186Bv,	31-mer 5'-	G A T A C C C T C T T C C A G G T T G C C A
2 non-sense	5: 187Bv,	31-mer 5'-	A C C T G T T G G A A A G A G G T A T C G C C A G G A
3 sense	6: 188Bv,	23-mer 5'-	A A C A T T C A G G A A T T T C A A C C C A
3 non-sense	7: 189Bv,	23-mer 5'-	T T T C C T G A A A T G T T T C A A C C C A
4 sense	8: 190Bv,	23-mer 5'-	T T A A G A A C A T C A G C T T C C C A A
4 non-sense	9: 191Bv,	23-mer 5'-	A G C T G A T G T T C T T A A T G G T T C C C A
5 sense	10: 192Bv,	23-mer 5'-	G G A C C A T G C A A A C T T C A A T A C C A
5 non-sense	11: 193Bv,	23-mer 5'-	A G T T T C C C A T G G C C C C C A T C A
6 sense	12: 194Bv,	23-mer 5'-	T T T C C C T C A G G C C C C C T T C A A
6 non-sense	13: 195Bv,	23-mer 5'-	A G G C C T G A G G G A A A G C T G A T C C T T
7 sense	14: 196Bv,	24-mer 5'-	T G A A G G A T C T G G A G G C C T G G A A C
7 non-sense	15: 197Bv,	24-mer 5'-	C C C T C C A G A T C C C T T C A A T G T T C
8 sense	16: 198Bv,	24-mer 5'-	G G C A A C T G G T G A T G C C A T C C A T
8 non-sense	17: 199Bv,	24-mer 5'-	C C A T C A C C A G T T G C C A T C C T T
all non-sense	18: 200Bv,	15-mer 5'-	C A T G C C A T C C G T A G

FIG. 3

1 (A-C)

GGTGTGTTTAATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCGACTGTTCAAG 60

G V F N Y E T E T T F S V I P A A R L F K 20

9 (A-G) 2 (A-C) 2 (A-C)

GCCTTTATCCTTGATGGCGATAACCTCTTTCCAAAGGTTGCACCCCAAGCCATTAGCAGT 120

A F I L D-G G D N-T L F P K-Q V A P Q A I S S 40

3 (GA-TC) 7 (AA-TC) 4 (G-C) 6 (GA-TC)

GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAAGAAGATCAGCTTTCCCGAA 180

V E N I E-S G N-S G G P G T I K K-N I S F P E-S 60

5 (CA-TG)

GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCACACAAACTTCAAA 240

G L P F K Y V K D R V D E V D H T-A N F K 80

TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC 300

Y N Y S V I E G C P I G D T L E K I S N 100

10 (GAG-CAC) 8 (CCC-TGG)

GAGATAAAGATAGTGGCAACCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC 360

E I K I V A T P-G D G G S I L K I S N K Y 120

CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC 420

H T K G D H E V K A E Q V K A S K E M G 140

GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCTACAATAA 480

E T L L R A V E S Y L L A H S D A Y N stop 159

FIG. 4

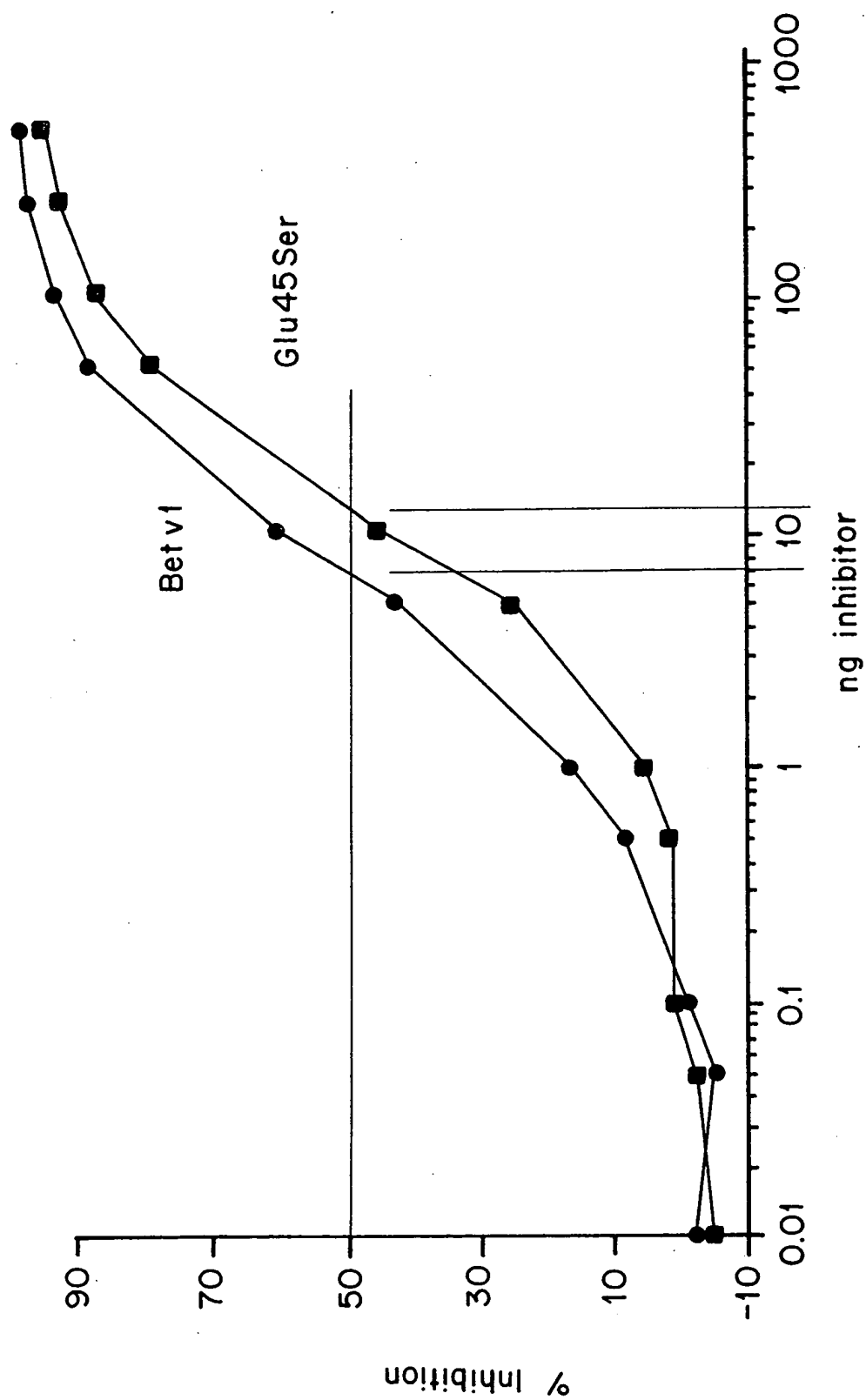


FIG. 5

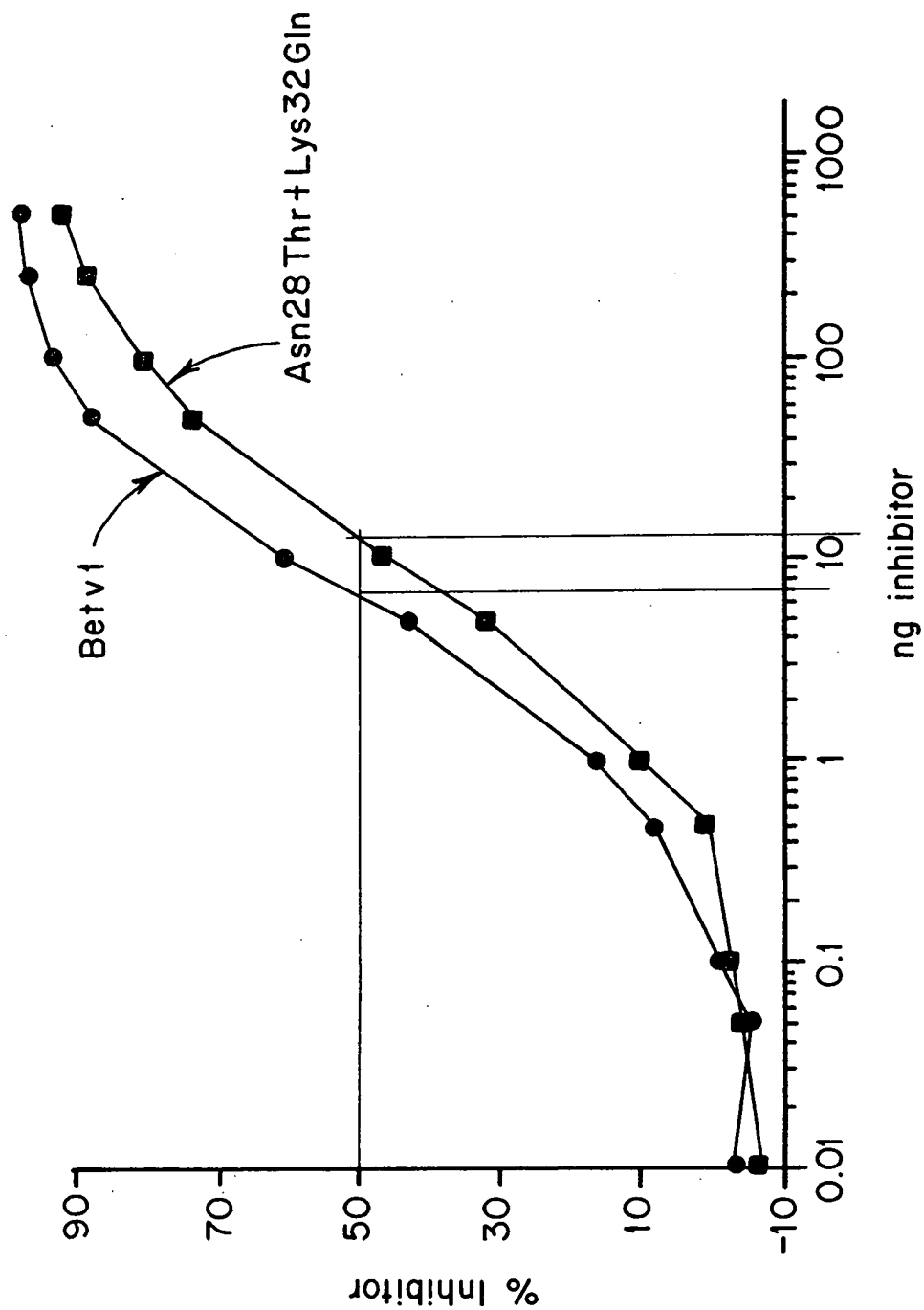


FIG. 6

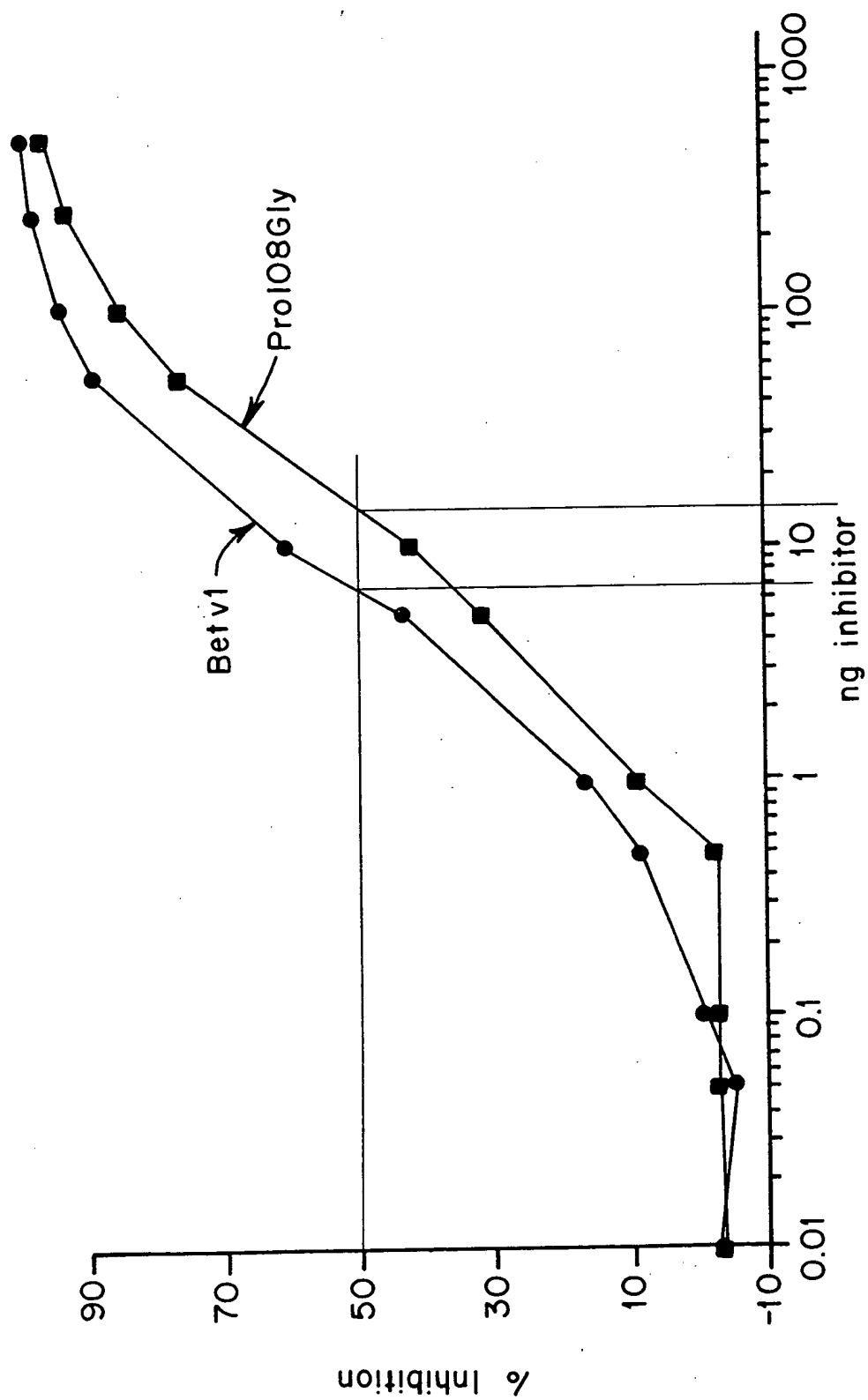


FIG. 7

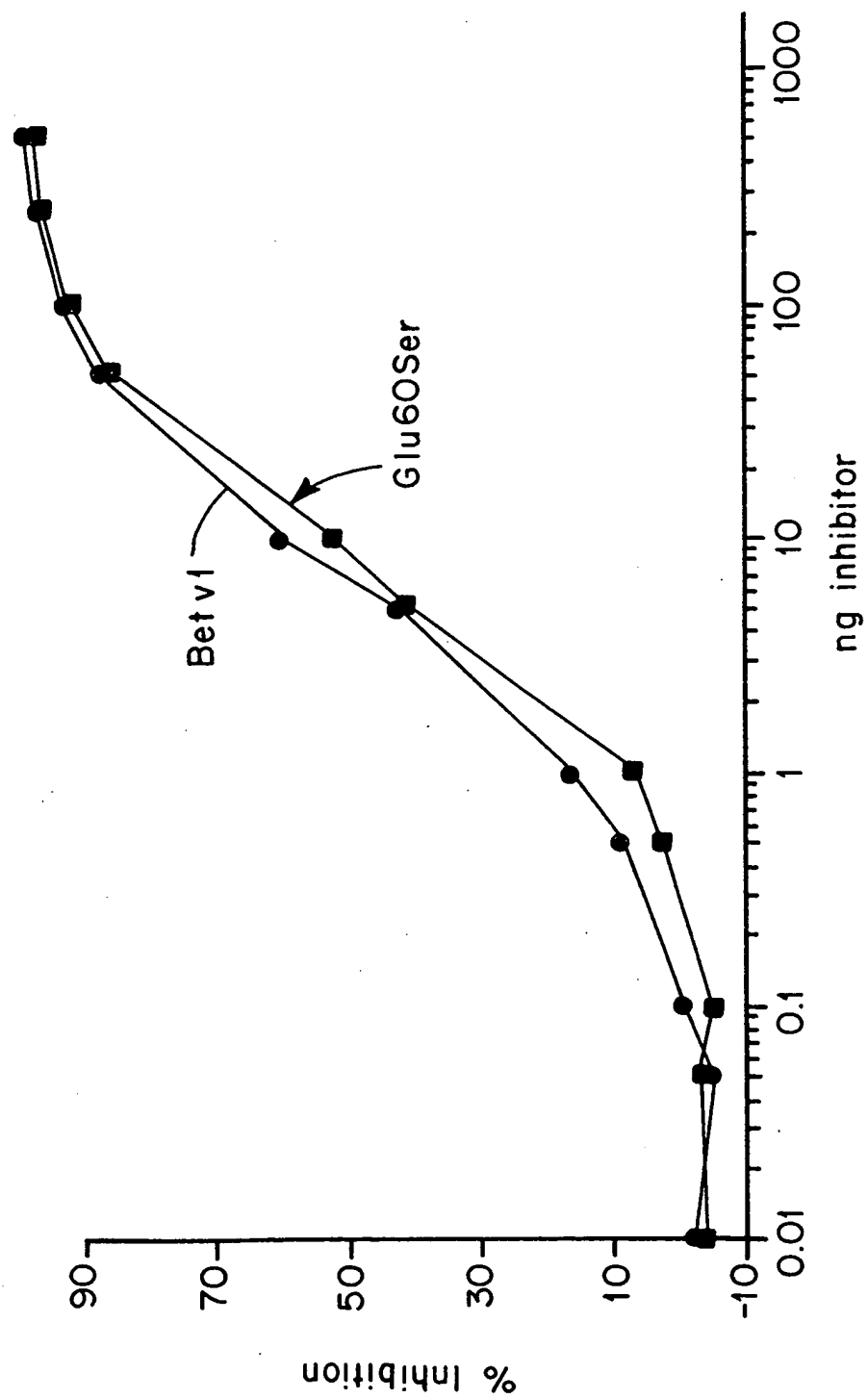


FIG. 8A

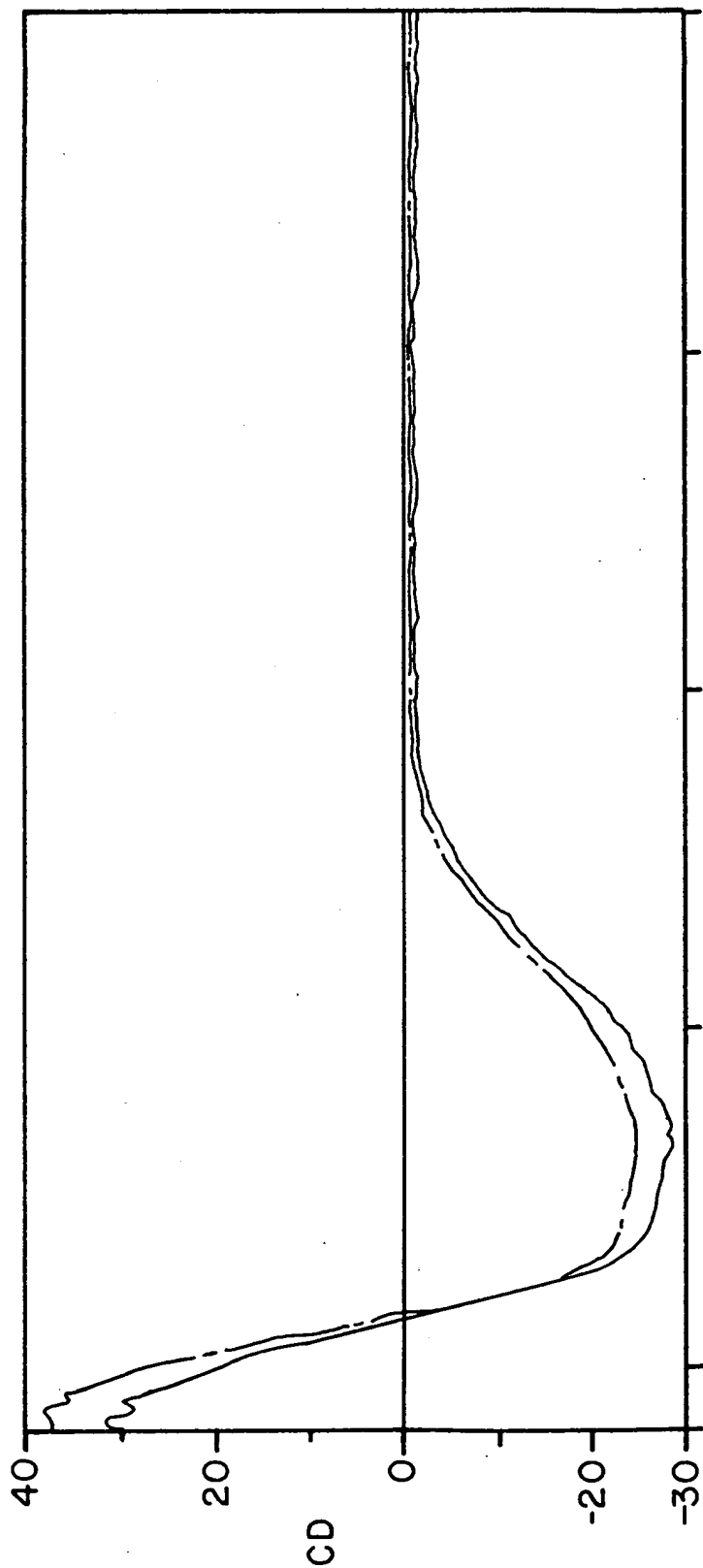


FIG. 8B

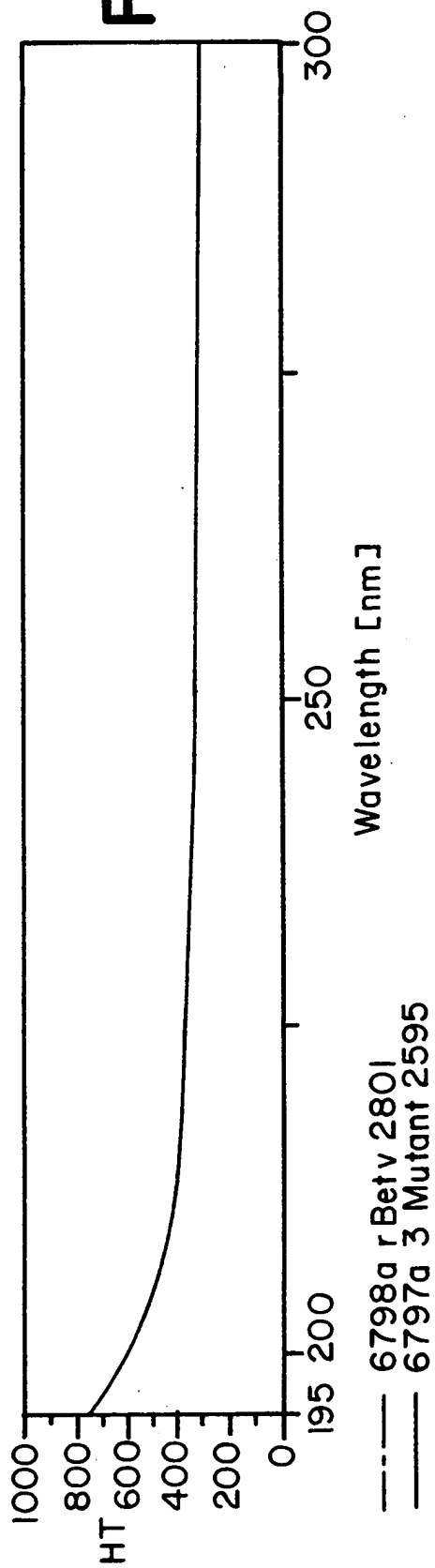
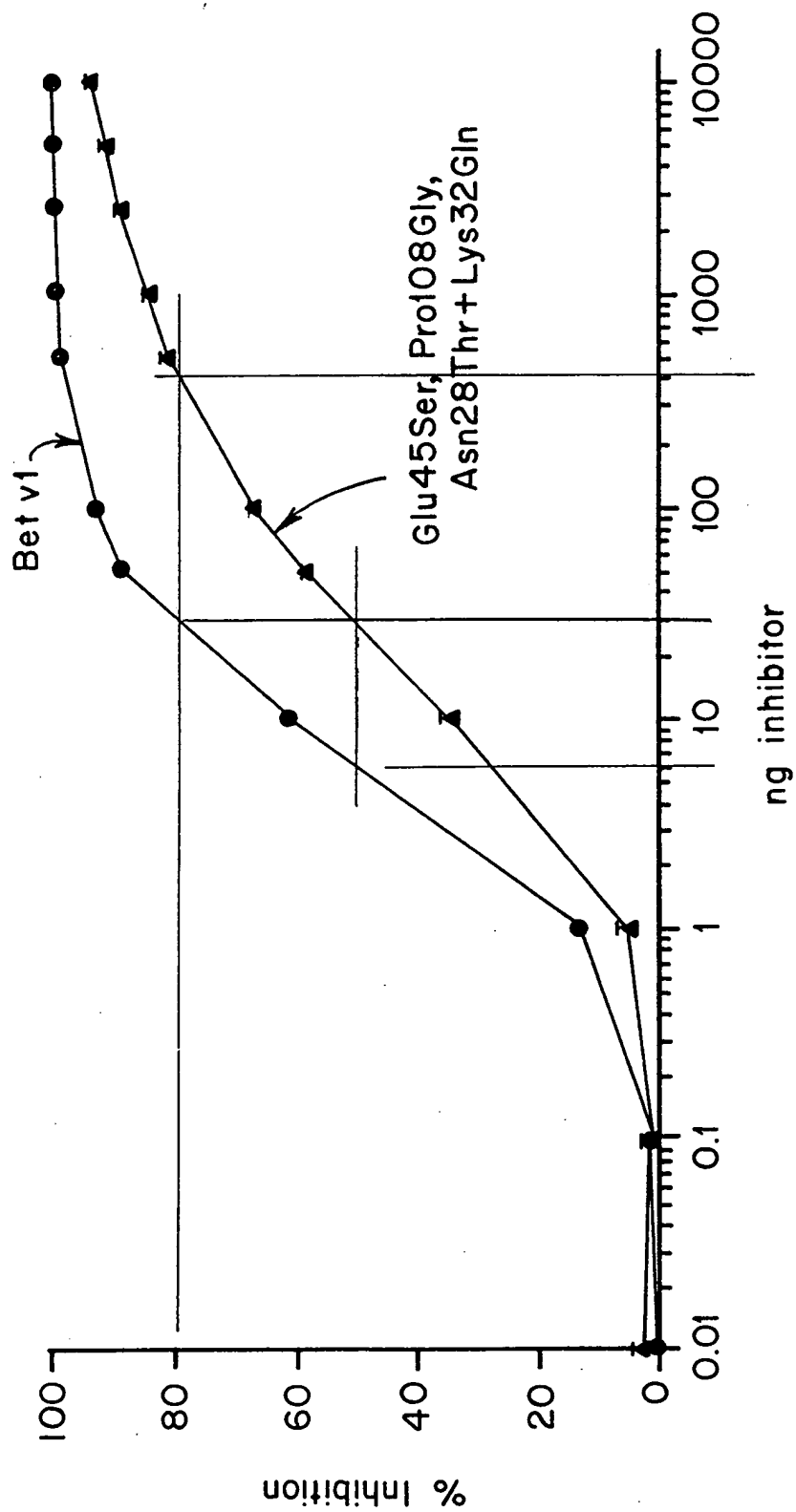


FIG. 9



APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
DRAFTSMAN		

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FIG. 10A



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FIG. 10B

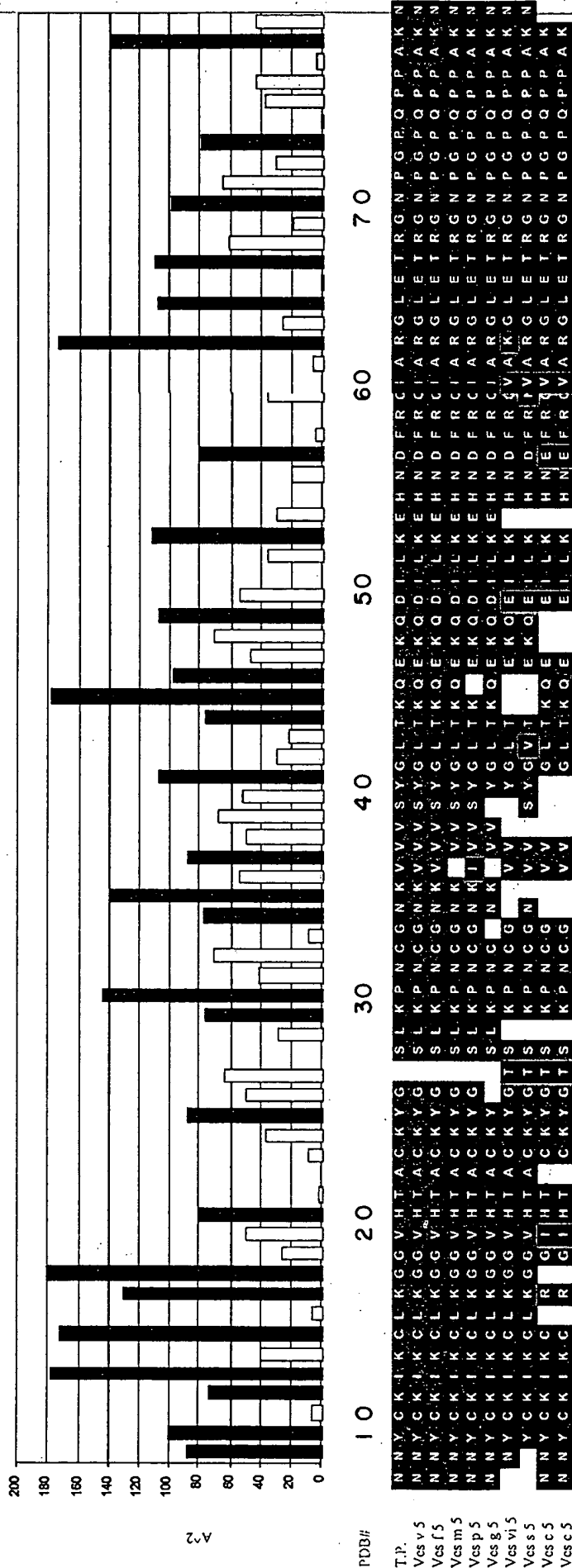
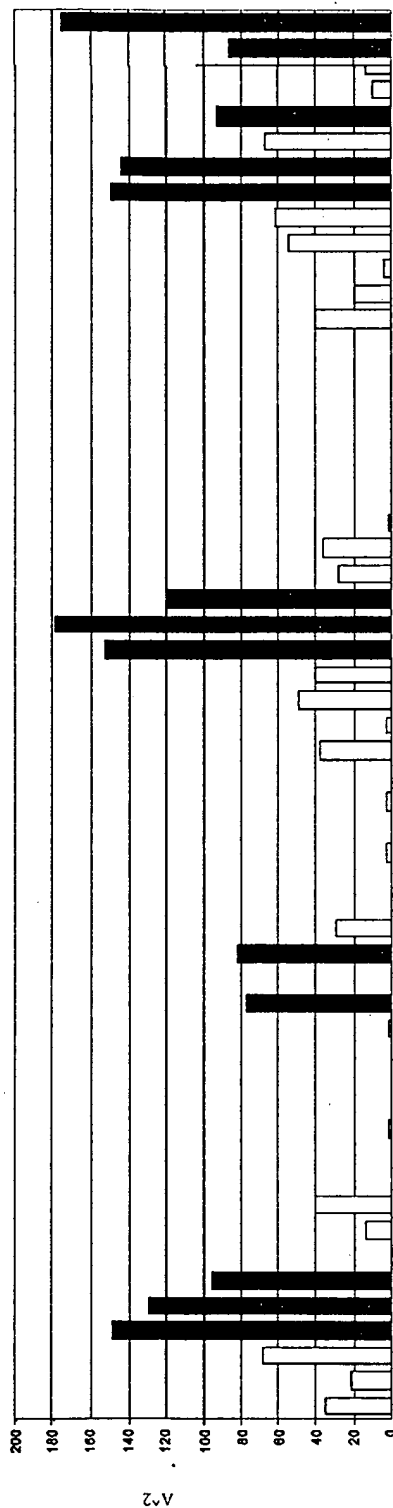


FIG. 10D



PDB#:

T.P.
Vcs v 5
Vcs f 5
Vcs m 5
Vcs p 5
Vcs g 5
Vcs vi 5
Vcs s 5
Vcs c 5
Vcs c 5

	160	170	180	190	200	120																																																	
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K	W	H	K	H	Y	L	V	C	N	Y	G	P	S	G	N	F	K	N	E	E	L	I	T	K
G	N	D	F	L	K	T	G	H	Y	T	Q	M	V	W	A	N	T	K	E	V	G	C	G	S	I	K	Y	I	Q	E	K</																								

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FIG. 11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants.
 Mutated nucleotides underlined.

Ves v 5 mutant 1 (K72A)

Ves v 5 sense	5'-	A C C A C A G C C C T C C A G C G A A G A A T A T G A A A A A T T T G G T A T G G A	3'
Ves v 5 non-sense	3'-	T G G T G T C G G A G G T C G G C T T C T T A T A C T T A A C C A T A C C T	5'
sense primer	5'-	C C A G C G G C T A A T A T G A A A A T	3'
non-sense primer	3'-	G T C G G A G G T C G C C G A T T A T A C	5'

Ves v 5 mutant 2 (Y96A)

Ves v 5 sense	5'-	G G C T A A T C A A T G T C A A T A T G G T C A C G A T A C T T G C A G G G A T G	3'
Ves v 5 non-sense	3'-	C C G A T T A G T T A C A G T T A T A C C A G T G C T A T G A A C G T C C C T A C	5'
sense primer	5'-	T T A G T T A C A G T T C G A C C A G T	3'
non-sense primer	3'-	T T A G T T A C A G T T C G A C C A G T G	5'

FIG. 12

Oligonucleotide primers for the site directed mutagenesis of Ves v 5.

all sense

1 : XhoI start, 38-mer:

EcoRI

5'-C C G C T C G A G A A A G A A C A A T T A T T G T A A A A T A A A T G
L E K R N N Y C K I K

KaX2 cleavage site

amino terminus of Ves v 5

1 sense

1 : K72As

21-mer

5'-C C A G C G G C T A A T A T G A A A A T

1 non-sense

2 : K72Aa

21-mer

5'-C A T A T T A G C C G C T G G A G G C T G

2 sense

3 : Y96As

21-mer

5'-T G T C A A G C T G G T C A C G A T A C T

2 non-sense

4 : Y96Aa

21-mer

5'-G T G A C C A G C T T G A C A T T G A T T

all non-sense

7 : CT-pPICZaA, 21-mer

5'-A T T C A T C A G C T G C G A G A T A G G

FIG. 13

1 AACAAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA 60
1 N N Y C K I K C L K G G V H T A C K Y G 20

61 AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG 120
21 S L K P N C G N K V V V S Y G L T K Q E 40

121 AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG 180
41 K Q D I L K E H N D F R Q K I A R G L E 60

1 (K 7 2 A) (AAG- GCT)

181 ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGAAC 240
61 T R G N P G P Q P P A K N M K N L V W N 80

2 (Y9 6 A) (TA -GC)

241 GACGAGTTAGCTTATGTGCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT 300
81 D E L A Y V A Q V W A N Q C Q Y G H D T 100

301 TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT 360
101 C R D V A K Y Q V G Q N V A L T G S T A 120

361 GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGGGAAGATGAAGTGAAAGATTAT 420
121 A K Y D D P V K L V K M W E D E V K D Y 140

421 AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG 480
141 N P K K K F S G N D F L K T G H Y T Q M 160

481 GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAAATACATTCAAGAGAAATGG 540
161 V W A N T K E V G C G S I K Y I Q E K W 180

541 CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT 600
181 H K H Y L V C N Y G P S G N F K N E E L 200

601 TATCAAACAAAGTAA 612
201 Y Q T K stop 204

FIG. 14

